

Protocol for Reagent Preparation for Use in the Neutralizing Antibody Assay for HIV-1 in TZM-bl Cells (April 2020)

1.0 Introduction

The preparation and maintenance of key reagents used for the Neutralizing Antibody Assay for HIV-1 in TZM-bl cells is crucial for obtaining accurate and reproducible results. Reagents must be created and stored as per manufacturer's guidelines and must be used within pre-established expiration dates.

2.0 Definitions

GM: Growth Medium

DMEM: Dulbecco's Modified Eagle Medium

FBS: Fetal Bovine Serum

HEPES: N-2-Hydroxyethylpiperazine-N'-2-Ethanesulfonic Acid

DEAE-Dextran: Diethylaminoethyl-Dextran

SDS: Safety Data Sheet

COA: Certificate of Analysis

3.0 Reagents and Materials

Recommended vendors are listed. Unless otherwise specified, products of equal or better quality than the recommended ones can be used whenever necessary.

Growth Medium

DMEM, with L-glutamine, sodium pyruvate, glucose and pyridoxine,

Manufacturer: Gibco BRL Life Technologies

Sterile, store refrigerated at 4°C

Fetal bovine serum

Manufacturer: Hyclone / Tissue Culture Biologicals

Heat-inactivated 56°C for 30 minutes, 500 ml bottle, sterile. Store at -20°C. Once thawed, store at 4°C.

Gentamicin solution, 10 mg/ml

Manufacturer: Sigma

Sterile, store at 4°C

HEPES

Manufacturer: Gibco BRL Life Technologies
Sterile, store at 4°C

DEAE-Dextran, hydrochloride, average Mol. Wt. 500,000

Manufacturer: Sigma

Britelite Plus Reporter Gene Assay System

Manufacturer: Perkin Elmer Life and Analytical Sciences

15 ml conical polypropylene tubes

Manufacturer: Generic

50 ml conical polypropylene tubes

Manufacturer: Generic

4.0 Instrumentation

Recommended manufacturers are listed. Unless otherwise specified, products of equal or better quality than the recommended ones can be used whenever necessary.

Pipettor

Manufacturer: Drummond

Scale

Manufacturer: Mettler

4°C Refrigerator

Manufacturer: LabRepCo

–20°C Freezer

Manufacturer: Sci-Cool / LabRepCo

Low Temperature Freezer (at least –70°C)

Manufacturer: Thermo Labsystems

5.0 Protocol

5.1 Growth Medium

5.1.1. Complete GM consists of DMEM containing 10% heat-inactivated FBS, 50 µg gentamicin/ml, and 25 mM HEPES.

5.1.2 To make 500 ml of Complete GM, combine and mix in a sterile bottle:

435 ml DMEM

50 ml FBS

2.5 ml of gentamicin

12.5 ml of HEPES

5.1.3 Store the Complete GM at 4°C for up to 2 months (or to the earliest expiration date of any one of the constituent reagents, whichever comes first). Reagents used to prepare Complete GM must be labeled with the date that the sterile bottle is opened. FBS bottles must be labelled with the date removed from the -20°C freezer to thaw, and expiration date. FBS expires 6 months after thaw when stored at +4°C.

5.1.4 Before use in the assay, warm medium to 20°-37°C.

5.2 Antibiotic-free Growth Medium

5.2.1 Antibiotic-free GM consists of DMEM containing 10% heat-inactivated FBS and 25µM HEPES.

5.2.2 To make 500ml of antibiotic-free GM, combine and mix in a sterile bottle:

437.5 ml of DMEM

50 ml of FBS

12.5 ml of HEPES

5.2.3 Store the antibiotic-free GM at 4°C for up to two months (or to the earliest expiration date of any one of the constituent reagents, whichever comes first).

5.2.4 Before use in the assay, warm medium to 20°-37°C.

5.3 DEAE-Dextran

5.3.1 To prepare a 5 mg/ml solution, dissolve 2.5 gm of DEAE-Dextran in 500 ml of sterile water.

5.3.2 Create 10 ml aliquots in 15 ml sterile conical polypropylene tubes.

5.3.3 Store aliquots between -20°C to -80°C. Once thawed, store at +4°C - +8°C until it is exhausted.

5.3.4 Once the tube is thawed, label the date it was thawed and the date it was opened.

5.3.5 DEAE-Dextran used in the Neutralizing Antibody Assay Laboratory does not have an expiration date. According to the COA, the manufacturer recommends retesting every three years for stability from the Quality Release Date.

NOTE 1: It is important to note that conical tubes with DEAE-Dextran solution should not be placed into styro foam racks for storage until after contents are completely frozen. The freezing process

begins at the exposed part of the tube. Consequently the shielded bottom of the tube will crack rendering the contents of the vial unusable.

5.4 Britelite Plus Reporter Gene Assay System

NOTE 2: The lyophilized Britelite Plus substrate is not classified as hazardous.

*Bright Glo substrate solution from Promega and Britelite substrate solution from Perkin Elmer Life and Analytical Sciences are acceptable substitutes for Britelite Plus. Please follow manufacturer's guidelines for preparation and use. Britelite and Bright Glo are classified as hazardous. Personal Protective Equipment (PPE) is required when working with these reagents.

NOTE 3: All reagents must be stored according to manufacturer's specifications and within manufacturer's specified expiration dates. An up to date SDS and COA must be retained in the laboratory

- 5.4.1 Equilibrate the BritelitePlus Substrate Buffer bottles to room temperature (about 30 minutes).
- 5.4.2 Pour one bottle of the BritelitePlus Substrate Buffer Solution (515ml) into two amber bottles of lyophilized BritelitePlus Substrate Solution bottles.
- 5.4.3 Recap the amber bottles and slowly mix (to prevent bubbles) the contents of the bottles by inversion until the substrate is completely dissolved (about 1 minute).
- 5.4.4 Pour the contents of both of the amber bottles back into the BritelitePlus Substrate Buffer Solution bottle.
- 5.4.5 Distribute 42.5ml of solution into 50ml labeled conical polypropylene tubes and store at -20°C for 1 month or -80°C for 3 months (or until the expiration date of the reagent, whichever comes first). The tubes should be stored on a labeled rack/container in the freezer.
- 5.4.6 Thaw in a room temperature water bath in the dark prior to use.
- 5.4.7 After thawing, gently invert the tube slowly to mix the solution prior to use. Use within 60 minutes of thawing. Excess reagent may be stored at -20°C or -80°C and used a maximum of 10 freeze/thaw cycles. Mark the vial with the number of freeze thaw cycles and the volume (in ml) remaining in the tube before refreezing.

NOTE 4: Stability of reconstituted Britelite Plus is approximately >85% remaining signal after 8 hours at 20°C.